CHAPTER 6

CHARACTERIZATION OF THE LAXATIVE COMPONENT OF THE WHOLE LEAF EXTRACT OF ALOE FEROX

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6.1 Background

*Aloe ferox* products, such as the latex and gel, are used as creams for skin ailments and as treatment for a wide range of diseases including laxative (Steenkamp and Stewart, 2007). The main active constituents of the latex and the leaf exudate are anthraquinones, which are believed to be responsible for the laxative effects, while the polysaccharide rich mesophyll is a good source of fiber (Wichtl and Bisset, 1994; Steenkamp and Stewart, 2007; Grace et al., 2009). These anthraquinones include aloins A and B, barbaloin, isobarbaloin, aloe-emodin, resins, aloesin and their derivatives. Usually, the age and climatic condition under which the plant is growing contribute to its levels of polysaccharides and flavonoids contents (Reynolds and Dweck, 1999; Hu et al., 2003).

Information on the traditional uses of *Aloe* species in herbal medicine against many diseases have been well documented (Watt and Breyer-Brandwijk 1962; Speranza et al., 1986, 1990; Koyama et al., 1994; Crouch et al., 2006; Kambizi et al., 2007). In addition, some compounds isolated from *A. ferox* such as 1, 8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione (aloemodin), 1, 8-dihydroxy-3-methyl-9, 10-anthracenedione (chrysophanol) and 10-C-β-D-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9-anthracenone (aloin A) have been found to be active, against microbial infections (Kambizi et al., 2004). The crude extracts of the plant showed significant activity against some bacteria and fungi (Afolayan et al., 2002). Recently, we revealed the laxative and toxicological effects of the aqueous whole leaf extract of *A. ferox* in loperamide-induced constipated rats (Wintola et al., 2010a, b).
The laxative property of this plant is attributed to its anthraquinones content which is poorly absorbed from the gastro intestinal tract (GIT) by gut bacteria. This, according to Blumenthal et al. (1998) is however converted to aloe-emodin that is more readily absorbed by the organism. The laxative effect is also believed to take place due to water accumulation in the intestine via active sodium ion (Na⁺) transport (Ishii et al., 1990).

Despite the bulk of scientific evaluations on *Aloes*, most work have been on *Aloe vera* or *Aloe barbadensis* and *Aloe arborescens*. Reports on *A. ferox* have been mostly on its gel or exudate. No scientific information is available on the compounds responsible for the laxative properties of the whole leaf extract of *A. ferox*. This work therefore was aimed at providing information on the laxative component of the whole leaf extract of *A. ferox*. The present study was specifically designed to isolate and identify the laxative compound(s) in the whole leaf extract of the species.

### 6.2 Materials and Methods

**Preparation of the over-the-counter (OTC) laxative drugs**

Over-the-counter laxative (OTC) drugs; Senokot and Soflax (Reckitt Benckiser Pharmaceuticals (Pty) Ltd & Cipla Life Science (Pty) Ltd respectively were purchased from a pharmaceutical store in Alice, Eastern Cape Province, South Africa. Each laxative was ground into powder using a mortar and pestle. The powder was weighed, placed in an air-tight sample bottle and kept for 24 h in the refrigerator. Two milligram of each drug was dissolved in 2 mls of water, methanol, chloroform, dichloromethane and ethyl acetate, to determine their appropriate solvent of solubility. Senokot and sofllax were soluble in water, methanol and ethyl acetate but insoluble in dichloromethane, chloroform and hexane.
**Chemical used**

Silica gel 60 F<sub>254</sub> thin layer chromatography (TLC) plates and all solvents (analytical grades) were supplied by Merck, Chemicals (Pty) Ltd, Wadeville, Gauteng, South Africa. Factory coated PTLC-plates SIL G-50 UV 254 (20 cm x 20 cm) were supplied by Merck (Midland, South Africa).

**Collection of plant materials**

Fresh, mature whole leaves of *A. ferox* were collected and authenticated as described in chapter 3. The leaves were oven dried to constant weight at 40°C and pulverized.

**Sample preparation and extraction**

400 g of the plant material was extracted with methanol (2 L X 3 times) by shaking on an orbital shaker (Stuart Scientific Orbital Shaker, UK) for 24 h. The extract was filtered using a Buchner funnel and 70 mm Whatman No. 1 filter paper. The filtrate was concentrated to dryness at 40°C on a rotary evaporator (Laborota 4000-efficient, Heildolph, Germany) to yield 32.23 g as shown in Figure 6.1. The methanol extract (32.23 g) was dissolved in 100 ml of methanol, and then suspended in distilled water (150 ml). This was successively partitioned with n-hexane, ethyl acetate and butanol (Kambizi et al., 2004).

**Isolation and fractionation of laxative compounds**

The active ethyl acetate fraction (3.45 g) was selected based on the result from the TLC plate after spotting the four fractions. This was subjected to fractionation on a column chromatography packed with silica gel. The crude material was dissolved in 10 mls of methanol.
and adsorbed into silica gel (Kiessl gel 60, 0.063-0.200 mm). The silica-extract was allowed to dry for 2 h before packing into the column. The column was eluted stepwise with solvent of increasing polarity ranging from a mixture of \( n \)-hexane: dichloromethane (0 - 100\%) to Dichloromethane: ethyl acetate (95 to 100\%) and ethyl acetate: methanol (50 to 95\% methanol), the solvent were eluted until it ran clear of the column. Eluted fractions of 12 mls each were collected successively and numbered 1-139.

Separation of compounds in the plant by thin-layer chromatography

Approximately 5 µL of the fractions were spotted on TLC plates and developed in benzene/ethanol/ammonium hydroxide (BEA, 90:10:1 v/v/v), chloroform/ethyl acetate/formic acid (CEF, 50:40:10 v/v/v) and ethyl acetate/methanol/water (EMW, 40:5:4.5 v/v/v). After developing, the TLC plates were allowed to dry and plates were examined under UV lamp at 254 and 365 nm (Chromato-Vue carbinet, CA9778 USA). The chromatograms were sprayed with vanillinic reagent, in an effort to detect the maximum number of compounds in the fractions. Fractions containing the same compounds as determined by the TLC plates were combined and concentrated into 6 fractions. Fraction 1 was chromatographed on a silica gel column eluted with \( n \)-hexane-dichloromethane (0:100 v/v), while fractions 2 and 5 were eluted with dichloromethane-ethyl acetate (70:30 v/v). Potassium hydroxide have been used to justify the presence of anthraquinones in crude plant extracts (Sakulpanich and Gritsanapan, 2009), notwithstanding, the presence of a target compound in crude plant extracts can also be confirmed by comparing its \( R_f \) with that of a known reference drug. Hence, the crude extract was co-chromatographed with the reference laxative drugs and the \( R_f \)s of the compounds which did not differ significantly from those of the standards were pooled together for further analysis.
**Preparative TLC**

Fractions of compounds were dissolved in small amount of chloroform (1 ml) and applied in a band across the preparative TLC plate (Silica gel 60 F<sub>254</sub>) leaving a margin of 1 cm. Factory coated PTLC-plates SIL G-50 UV 254 (20 cm x 20 cm) were supplied by Merck (Midland, South Africa). on both sides of the plate, the plate was then developed in chloroform/ethyl acetate/formic acid (CEF 5:4:1, v/v/v) and the bands visualized under UV light (254 and 365 nm). The bands were scrapped and collected into separate vials. The components in the vial were dissolved in small quantity of chloroform using magnetic stirrer to stir for 30 min to remove the adsorbed silica from the compound. The sample was filtered with Whatman No 1 filter paper (70 mm) on a sintered glass funnel. The filtrate was concentrated under a steam of cold air.

**Spectroscopy**

UV, IR and NMR techniques were used for the analysis and identification of the compounds isolated from the whole leaf extract of *A. ferox*. Deuterated chloroform (CDCl<sub>3</sub>) was used as a choice solvent because of its ability to dissolve a wide range of compounds and its easy volatility. The TLC chromatograms were viewed under UV lamp at 254 and 365 nm (Chromato-Vue carbinet, CA9778 USA). Perkin Elmer 2000 Fourier-transform infrared (FTIR) spectrometers was used to run the IR of the isolates. <sup>1</sup>H (400MHZ) and <sup>13</sup>C (100.60 MHZ) NMR were recorded on a Bruker AMX 400 and 600 instrument with chemical shift reported in ppm relative to internal standard. 2D NMR spectra were recorded on the same instrument using field gradient BBI (inverse) probe. The pure samples were weighed and dissolved in 2 ml deuterated chloroform (CDCl<sub>3</sub>) used for NMR. The samples were then pipetted into NMR tube with the
aid of a Pasteur pipette and run on NMR spectrometer using XNMR top spin at the Organic Chemistry Laboratory of Rhodes University, Grahamstown, South Africa.

6.3 Results and Discussion

*Plant extraction*

The bulk extractions of *A. ferox* whole leaf extract with methanol (400 g) yielded 32.23 g.
Fresh whole leaf plant material collected and oven dried (400 g)

Extracted with methanol

Methanol extracts

Concentrated on rotary vacuum evaporator

Dried methanol extract (32.23 g)

Suspended and fractionated with n-hexane

n-hexane fraction (3.03 g)

Water fraction

Fractionated with ethyl acetate

Ethyl acetate fraction (3.45 g)

Water fraction

Fractionated with butanol

Butanol fraction (2.05 g)

Water fraction (2.53 g)

Figure 6.1: Schematic procedure for the solvent partitioning of the crude extract from *A. ferox*
Isolation and identification of laxative compounds

TLC plate in Figure 6.2 in this order: (a) The partition fractions showing hexane (H); ethyl acetate (E); butanol (B); water (W). The TLC was eluted with ethyl acetate/methanol/water (EMW, 40:5:4.5 v/v/v) and chloroform/ethyl acetate/formic acid (CEF, 50:40:10 v/v/v).

Figure 6.2: TLC plate showing the partitioned fractions

These were further spotted with the OTC drugs in this order: Hexane (H); senokot in methanol (Mk); ethyl acetate (E); soflax in methanol (Ml); butanol (B); senokot in ethyl acetate (Ek); water (W); soflax in ethyl acetate (El) and elute with ethyl acetate/methanol/water (EMW, 40:5:4.5 v/v/v) and chloroform/ethyl acetate/formic acid (CEF, 50:40:10 v/v/v) as shown in Figure 6.3.
Figure 6.3: TLC chromatogram of fractions and OTC drugs.
Figure 6.4: Schematic presentation of the isolation of compounds from ethyl acetate fractions of the whole leaf of *A. ferox*.
Combined fraction 1 obtained from (Hexane/DCM; 100:0, v/v) was loaded on small CC over silica gel to give 29 sub-fractions. Sub-fraction 25 distinct itself from the rest by producing a blue florescence compound (365 nm), with an R$_f$ of 0.42 which is similar to one of the components of the OTC drug. This compound was purified by using over preparative TLC to give a compound weighing 0.022 g.

Fraction 2 obtained from DCM /EtOAC (90:10, v/v) were co-spotted with the OTC drugs and subjected to column chromatography packed with silica gel. A total of 42 sub-fractions of 12 ml each were collected. This was plated with the OTC drug and sub-sub-fractions 2 and 6 were observed to have similar characteristic. Sub-sub-fractions 2 and 6 eluted with DCM /EtOAC (70:30, v/v) were co-spotted with the OTC drugs and run with BEA, CEF and EMW. It was then combined and further purified on preparative TLC to give a yellow compound yield 0.032 g. The R$_f$s of the blue and yellow compounds (0.420 & 0.831) were similar to the standard laxative drug (Senokot & Soflax) as shown in Figure 6.5. This suggests that there could be some similar compounds present in the fractions and OTC drugs. Whilst studies is in progress for the determination of the laxative activity of these fractions on constipated rats, co-chromatography of the fractions with OTC drugs was a quick and cheaper method of eliminating non-laxative fractions.
Figure 6.5a: Fraction 25 showing the blue compound co-spotted with OTC drug

(a) CEF

(b) EMW

Figure 6.5b: Yellow fractions co-spotted with the OTC drugs; senokot in DCM; senokot on MeOH; fraction 2; soflax in MeOH; fraction 6; soflax in EtOAc and eluted with CEF & EMW.
Spectroscopy

The FT-IR result shows different absorption spectra, each absorption, depicting a functional group which is basically the functional group of the compounds. The IR spectrum of the yellow compound showed absorption peaks at 3418, 1632, 1618, 1400, 1261 and 1105 cm\(^{-1}\) (see appendix). The blue compounds, have similar absorption at this IR region suggesting OH functional group absorption. The absorption peaks at 1617 and 1621 is also common to the two compounds and are carbonyl absorption of carboxylic and not of aldehyde or ketone.

Furthermore, the absorption at 2922.96-2853.25 cm\(^{-1}\) are C-H stretching vibration while those at 1457.79 –1377.85 cm\(^{-1}\) are C-H bending. The C-O stretching vibration are at 1088.35-1037.23 cm\(^{-1}\), while the prominent peaks at 1714.68 – 1626.15 cm\(^{-1}\) are C=O stretching. Compounds with OH groups are biologically active and the more the OH groups in the compound, the more the biological activities (Dosumu et al., 2010). Crude extract, a mixture of compounds is likely to have more OH groups than the isolated pure compound(s), hence high biological activities. Phenolic compounds inhibition activities have also been found to be proportional to the number of OH group present in the compound (Friedman et al., 2003; Dosunmu et al., 2010). Though, the structure of the isolated compounds could not be fully elucidated, but the presence of OH and C=O functional groups were established, which were tagged to the bioactivities of the plant.

The results of the extensive chromatography and NMR investigation showed a chemical shift between δ 6.5-7.8 which are those of aromatic protons, while the chemical shift at δ 12.08 is that of hydroxyl proton of an acid (OH-COOH). There is instability in the compound mixtures of the whole leaf extract of A. ferox, which makes the total elucidation of the compounds difficult, probably due to insufficient fractions, problems like impurities in the compound as reflected in the spectra e.g. \(^{13}\)C, \(^{1}\)H and 2D NMR. The \(^{13}\)C-NMR shows a resume
of about δ 210 which the carbon of a non shielded carboxyl (C=O). This was as shown in Figure 6.12- 6.15.

Although the isolation and purification of the active laxative compound(s) from the ethyl acetate extract of *A. ferox* whole leaf was not successful, a study undertaken by Kambizi et al. (2004) reported three compounds isolated from this species namely; 1, 8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione (aloe-emodin), 1, 8-dihydroxy-3-methyl-9, 10-anthracenedione (chrysophanol) and 10-C-β-D-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9-anthracenone (aloin A). Aloe emodin and aloin have been reported to be associated with most biological medicinal activities including laxative action (Van Wyk et al., 1997; Kambizi et al., 2004). These compounds may be responsible for the observed laxative activity investigated in this study.
References


Briza Publications, Pretoria.


